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# The role of p53 and the CD95 (APO-1/Fas) death system in chemotherapy-induced apoptosis

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**ABSTRACT.** To explore the pathway of p53 dependent cell death, we investigated if p53 dependent apoptosis following DNA damage is mediated by the CD95 (APO-1/Fas) receptor/ligand system. We investigated cell lines of solid human tumors upon treatment with clinically relevant chemotherapeutic drugs known to act *via* p53 accumulation. Treatment with these cytotoxic drugs led to an upregulation of both, the CD95 receptor (CD95) and the CD95L (CD95L). Induction of the CD95L occurred in p53 wild-type (wt), p53 mutant (mt) and in cell lines lacking p53 altogether (p53<sup>-/-</sup>). Thus, the regulation of the CD95L in response to chemotherapeutic drugs clearly involves p53 independent mechanisms. Most importantly, upregulation of CD95 occurred only in cell lines with wild-type p53, thereby strongly increasing the responsiveness towards CD95 mediated apoptosis. Thus, upregulation of the CD95 receptor seems to be dependent on intact wild-type p53. Apoptosis was mediated by cleavage of the receptor proximal caspase, caspase-8 (FLICE/MACH). Caspase-8 cleavage was observed, independent of the p53 status of the tumor cells and irrespective whether or not apoptosis was dependent on the CD95 system. Hence, additional effector pathways besides CD95/CD95L signaling are likely to contribute to drug-induced apoptosis.

**Keywords:** apoptosis, CD95 (APO-1/Fas), p53, FLICE, cancer therapy, drug sensitivity and -resistance.

## INTRODUCTION

p53, the "cellular gatekeeper for growth and division" [1], is the most commonly mutated gene in human cancer, and its inactivation contributes not only to tumor progression but also to resistance of cancer cells to chemotherapy. Biologically, the best known activities of p53 are cell growth arrest and induction of apoptosis. p53 is a component of the pathways activated in response to DNA damage, for example by chemotherapeutic agents. Cell cycle arrest at the G1 and G2 checkpoints prior to DNA replication and mitosis aids the DNA repair processes and prevents mutations and aneuploidy, whereas apoptosis can be considered as a mechanism to rid the organism of cells with severely damaged DNA. The mechanisms of p53-dependent apoptosis are still not completely elucidated. The presence of functional wild-type p53 is closely coupled with efficient induction of CD95-mediated apoptosis in many [2, 3] but not all [4] cell types. Based on our observation [3] that the CD95 receptor was only upregulated in cancer cells carrying wt p53 following DNA damage and on the fact that forced overexpression of wt p53 can stimulate CD95 gene transcription [5], we investigated if the CD95 system is activated/regulated by p53 in a variety of human solid cancer cell lines.

## MATERIALS AND METHODS

**Cell lines:** 1. HepG2 cells (human hepatoblastoma) only expressing small amounts of wild-type p53; 2. Huh7 cells (hepatocellular carcinoma) shown to express mutant p53; 3. Hep3B cells deficient of p53; 4. Hs746T gastric cancer cells expressing wild-type p53 and 5. HT29 colon cancer cells with mutated p53. The CD95 receptor was stimulated with the monoclonal antibody IgG 3 anti-APO-1 (100 ng/ml) [6]. Immunodetection of FLICE was performed using the mouse anti-FLICE monoclonal antibody C15 recognizing the FLICE active subunit p18 [7]. Cell surface expression of the CD95 receptor was assessed by FACScan. Anti-APO-1 (IgG3,  $\kappa$ ) was used as purified biotinylated antibody. Quantum Red streptavidin (Sigma, Munich, Germany) was used as secondary reagent for indirect immunofluorescence. PCR analysis of the CD95 ligand was performed as described [3].

## RESULTS

### *Induction of the CD95 receptor by anti-cancer drugs*

Treatment with 5-fluorouracil, methotrexate, mitomycin, cisplatin, mitoxantrone, doxorubicin, etoposide, cyclophosphamide and bleomycin led to upregulation

of the CD95 receptor in cell lines containing wild-type p53. In contrast, no induction or only weak induction of the CD95 receptor was observed in cells with mutant p53, and in cells lacking p53. Treatment of cell lines carrying wild-type p53 with diverse chemotherapeutic drugs 48 hours prior to addition of the agonistic apoptosis inducing antibody IgG3-anti-APO-1 resulted in significant enhancement of apoptosis compared to anti-APO-1 treatment alone or treatment with the anti-cancer drug alone. Not only an additive but a synergistic effect in induction of apoptosis by anti-cancer drugs in combination with anti-APO-1 was observed with all the drugs and wild-type p53 containing cell lines tested.

#### **Induction of the CD95 ligand (CD95L) by anti-cancer drugs**

We found that anti-cancer drugs with different mechanisms of action upregulated CD95L mRNA in hepatoma-, gastric-, and colon cancer cell lines independent of the p53 status of the tumor cells.

#### **Different anti-cancer drugs induce cleavage of caspase-8 (FLICE/MACH $\alpha$ 1/Mch5)**

FLICE cleavage into the cleavage intermediates p43, p41 and the active subunit p18 could be demonstrated in Hs746T cells following treatment with doxorubicin and in HT29 cells following treatment with 5-fluorouracil. Activation of FLICE was not dependent on the p53 status of the cell. Furthermore, FLICE cleavage did not correlate with induction of CD95.

#### **DISCUSSION**

The data in this paper show that clinically relevant concentrations of diverse anti-cancer drugs induce CD95 receptor expression in hepatoma, gastric cancer-, and colon- cancer cell lines. Thus, chemotherapy may sensitize tumor cells by upregulation of death regulators such as the CD95 receptor. Most notably, CD95 upregulation seems to be dependent on functional wild-type p53. Treatment with chemotherapeutic agents failed to induce the CD95 receptor in tumor cells with mutated or deficient p53. This would argue for a differential regulation of the CD95 gene by wild-type and mutant p53. A critical question is whether p53-induced apoptosis is functionally dependent on the induction of the CD95 gene. With the identification of killer/DR5 [8] as another pathway of p53-dependent apoptosis following DNA damage, it is evident that p53-dependent apoptosis is not solely mediated by the CD95 system. However, the observation that p53-dependent cell death following DNA damage is mediated by TNF-R family members could have significant implications for manipulating apoptosis and cancer therapy in the future. Furthermore, treatment with cytotoxic drugs not only lead to upregulation of the CD95 receptor but also of the CD95 ligand in hepatoma-, gastric-, and colon cancer cell lines. CD95L upregulation in response to chemotherapeutic drugs has also been shown for leukemias [9].

The regulation of CD95L clearly involved p53 independent mechanisms. As our blocking experiments with F(ab')<sub>2</sub>-anti-APO-1 antibody fragments did not show complete blocking of apoptosis, other death systems additional to the CD95 system might play a role in drug-induced apoptosis; these systems may include TNF-RI, TNF-RII, TRAIL-RI-4. This is supported by our data on expression of caspase-8 in response to cytotoxic treatment. Cleavage of caspase-8 was observed even when the CD95 pathway was not used. Considerations of drug resistance in the future, therefore, will have to incorporate testing the functionality of the CD95 and other death systems.

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#### **REFERENCES**

1. Levine A J. 1997. p53, the cellular gatekeeper for growth and division. *Cell* 88: 323.
2. Tanura T, Aoyama N, Saya H, Haga H, Futami S, Miyamoto M, Koh T, Ariyasu T, Tachi M, Kasuga M, Takahashi R. 1995. Induction of Fas-mediated apoptosis in p53-transfected human colon carcinoma cells. *Oncogene* 11: 1939.
3. Müller M, Strand S, Hug H, Heinemann E M, Walczak H, Hofmann W J, Stremmel W, Krammer P H, Galle P R. 1997. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. *J. Clin. Invest.* 99: 403.
4. Fuchs E J, McKenna K A, Bedi A. 1997. p53-dependent DNA damage-induced apoptosis requires Fas/APO-1-independent activation of CPP32beta. *Cancer Res.* 57: 2550.
5. Owen-Schaub L B, Zhang W, Cusack J C, Angelo L S, Santee S M, Fujiwara T, Roth J A, Deisseroth A B, Zhang W W, Kruzel E, Radinsky R. 1995. Wild-type human p53 and a temperature-sensitive mutant induce FAS/APO-1 expression. *Mol. Cell Biol.* 15: 3032.
6. Dhein J, Walczak H, Baumler C, Debatin K M, Krammer P H. 1995. Autocrine T cell suicide mediated by APO-1/(Fas/CD95). *Nature* 373: 438.
7. Scaffidi C, Medema J P, Krammer P H, Peter M E. 1997. Two FLICE isoforms, caspase-8/a and caspase-8/b, are recruited and activated by the CD95 death-inducing signaling complex (DISC). *J. Biol. Chem.* 272: 26953.
8. Wu G S, Burns T F, McDonald E R, Jiang W, Meng R, Krantz I D, Kao G, Gan D D, Zhou J Y, Muschel R, Hamilton S R, Spinner N B, Markowitz S, Wu G, El-Deiry W S. 1997. KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene [letter]. *Nat. Genet.* 17: 141.
9. Friesen C, Herr I, Krammer P H, Debatin K M. 1996. Involvement of the CD95 (APO-1/Fas) receptor/ligand system in drug-induced apoptosis in leukemia cells. *Nature Medicine* 2: 574.